

1. The first step in the process is to identify the problem or issue that needs to be addressed. This involves gathering information and understanding the context of the problem.

2. Once the problem is identified, the next step is to define the objectives and goals of the project. This helps to clarify what needs to be achieved and provides a clear direction for the team.

3. The third step is to develop a plan or strategy to address the problem. This involves breaking down the problem into smaller, manageable tasks and determining the resources needed to complete each task.

4. The fourth step is to implement the plan. This involves putting the strategy into action and monitoring progress to ensure that the project is on track.

5. The final step is to evaluate the results of the project. This involves assessing the outcomes against the objectives and goals and identifying any areas for improvement.

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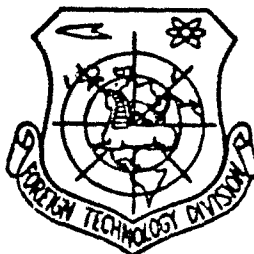
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BIOLOGICAL SENSORS

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TITLE: LECTURES ON NEW MODELS OF SENSORS: LECTURE NO. 9.

BIOLOGICAL SENSORS

AUTHOR: ZHANG GUOXIONG

Biological sensor transmitting devices are created by a combining of fixed biological material and an appropriate transducer device. This transducer takes biochemical signals and converts them into fixed amounts of electrical signal.

Biological molecules possess properties such that they can distinguish a single chemical compound or a small family of chemical compounds and combine with them. This type of distinguishing and combining possesses very good selectivity. If one takes the classes of biological materials that are used, biological sensors can be divided into enzyme sensors, microbiological sensors, immune sensors, cell and tissue sensors, and so on.

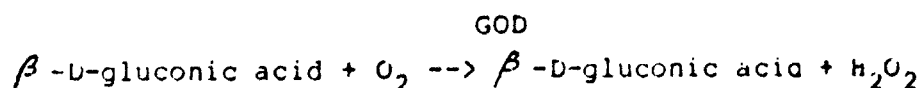
As far as requirements to prepare biological sensors are concerned, it is necessary to take biological materials and, using physical methods, fix them into a basic substance or substrate membrane or gel. It is also possible, by chemical methods, to absorb them into or bond them onto a surface. It does not matter what method is used. It is necessary to make the biological material have a definite activity and a definite life.

Used as the transducer devices of biological sensors, there are electrical terminals or electrodes, field effect transistors, optical fibers, thermistors, as well as piezo-electric crystals and other differing models of a similar type.

I. ENZYME SENSORS

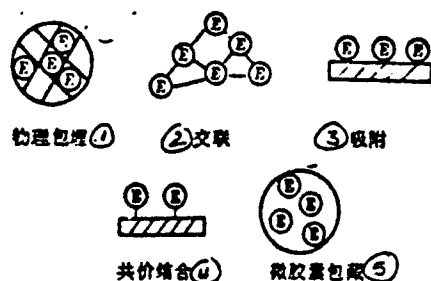
(I) Enzyme Electrodes

Enzyme electrodes are formed from a combination of fixed enzymes and electro-chemical measuring devices. The most familiar example is the glucose oxide enzyme electrode. Outside China, in the 1970s, it had already reached the stage of actual use. This electrode is principally formed from an enzyme membrane of fixed glucose oxide and an oxygen electrode. The glucose oxide enzyme (GOD) selectively catalyzes the reaction below:



This reaction consumes a fixed quantity of oxygen. It is possible to use oxygen electrodes to sensitively reflect the changes in oxygen. The results from this make it possible, from the reductions in the oxygen dissolved in the experimental liquids, to solve for the concentration of glucose. The test manufacture of oxygen electrodes that have high sensitivity, quick reaction times, good stability, and small residual current flows, as well as the test manufacture of fixed enzyme membranes with strong activities, long life, and good interference resistance properties are the keys to the properties of glucose sensors being good or bad. The author, in the experiments, used platinum wire to make the negative electrodes, and silver/silver chloride to make the positive electrodes. He used tetrafluoroethylene-hexafluoropropylene copolymer to make the gas permeable membrane. He test produced an oxygen electrode with excellent properties. When measuring gaseous oxygen, the electrodes, in the 1.0~25% range showed excellent linear reactions. The residual currents were smaller than 0.03%. The electrodes, from amounts of contained oxygen of 21% to 0.03%, only required 10 seconds to reach equilibrium. When temperature was constant, during continuous measurements for 500 hours, the drift was smaller than 1%. In the glucose sensor test-manufactured with the glucose oxide enzyme compound provided by the Shanghai Biochemical Research Institute, within ranges of 5×10^{-6} ~ 2×10^{-3} mol concentration, one saw linear reactions. Replicability and stability were both excellent. The enzyme membrane is capable of continuous use for 3000 iterations. When at 4°C and maintained there for 300 days, the activity was still capable of satisfying the requirements for measurements.

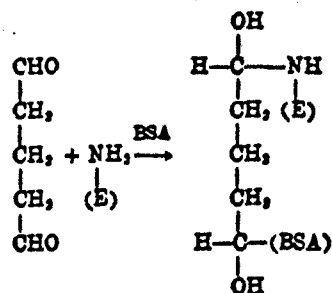
The technology of fixing enzymes has the several forms that follow: physical envelopment, the use of dual functions or multi-functional test agent coupling, absorption, covalent combining with non-water soluble carriers, and storage in tiny gelatin capsules. It is possible to use the figures below to show this in graphic form. In the figures, E stands for the enzyme.



(1) Physical Envelopment (2) Coupling (3) Adsorption (4) Covalent Bonding (5) Storage in Tiny Gelatin Capsules

The method of physical envelopment is to take monomers of a high polymer and, when they are combining, take the enzyme molecules and envelop or bury them in the lattice of the polymerizing substances. The most commonly used polymerizing substances are propenoic acid amine polymers. The next most common are silicone rubber, starch, and silica gel, as well as other similar substances. The advantages of the physical envelopment or burying method are that reaction conditions are moderate and it is appropriate and capable of being used with all the various types of enzymes. The disadvantages are that the free ions produced in the polymerization process will cause the enzymes to lose activity and the enzymes can easily leak away and be lost.

The method involving dual function test reagent coupling often makes use of Glutard aldehyde (GA) to make coupling agents. It reacts together with bovine serum albumin and enzymes to produce polymers that do not dissolve in water. The coupling reactions can be represented in the form below:



The types of enzymes that are fixed with methods of this type are very numerous. The disadvantage is that there are a number of enzymes that are sensitive to coupling agents and, in the coupling process, will lose activity.

Absorption refers to the adhering of enzymes to the surfaces of carrier substances. During it, there are no especially effective functions or activities to use in making covalent coupling. Among the absorption agents, there are glass, quartz, activated charcoal, silica gel, aluminum oxide, ion exchange resins, and other similar substances. The advantage of this method is that control is simple. It is not very destructive to enzymes. However, the absorption of the enzymes is related to pH, solvents, substrates, and temperature. When these factors change, it is easy to undo the absorption and cause the enzyme to flow away.

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Going through covalent coupling, one takes the enzymes and bonds them to the carrier bodies. After they are fixed, they will not, because of changes in pH ion strength, substrates, solvents, or temperature, reverse the reactions. Carriers can be divided into non-organic carriers such as porous glass, natural high polymers, and composite high polymers (such as nylon, propenoic acid amine polymers, ethanoic acid polymer derivatives, and other similar substances). When fixed, first the carrier substance activates. After that one uses coupling agents to couple the enzyme to the basic body. The reaction speed of biological sensors, their sensitivity, life, and other similar special properties, to a very great degree, depend on the manufacturing methods relying on fixation of enzymes. In order to

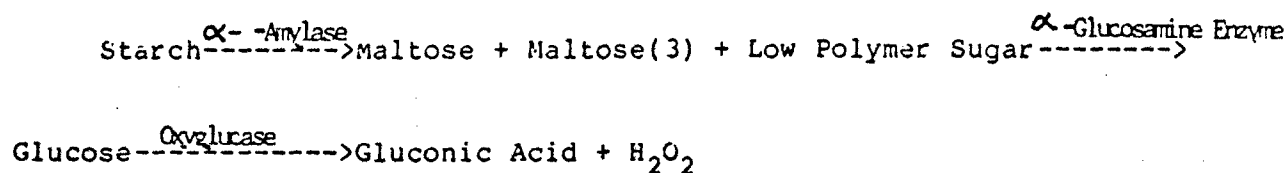
make biological sensors actually useful, it is necessary to carry out the best possible initial research on such factors as the permeability of substrates, the amount of enzyme, and other similar factors which have to do with fixation methods. As far as enzyme sensors for medical uses are concerned, it is also necessary to do research on stopping the contamination of blood fluid components. This is particularly true of the contamination of membranes by proteins and lipids.

Reports on a number of enzyme sensors are set out in Table 1. Their basic structure and glucose electrodes are the same. However, their detection devices will show some differences because of the materials participating in enzyme reactions.

2 测定对象	3 酶	4 检测电极	5 测定范围(mol) S
6 葡萄糖	15 葡萄糖氧化酶	O ₂	10 ⁻⁵ ~10 ⁻³
	16 葡萄糖氧化酶	Pt(H ₂ O ₂)	2×10 ⁻⁴ ~10 ⁻⁴
	17 葡萄糖氧化酶	pH	10 ⁻⁴ ~10 ⁻³
7 蔗糖	18 转化酶+葡萄糖氧化酶	O ₂	2×10 ⁻⁵ ~10 ⁻³
8 尿素	20 脲酶	NH ₃	10 ⁻² ~10 ⁻⁶
	21 脲酶	CO ₂	10 ⁻² ~10 ⁻⁴
	22 脲酶	pH	10 ⁻² ~10 ⁻³
9 尿酸	23 尿酸酶	O ₂	5×10 ⁻⁴ ~5×10 ⁻³
10 胆甾醇	24 胆固醇酯酶+葡萄糖氧化酶	O ₂	10 ⁻² ~10 ⁻³
11 L-氨基酸	25 L-氨基酸酶	Pt(H ₂ O ₂)	10 ⁻³ ~10 ⁻⁴
	26 L-氨基酸酶	O ₂	10 ⁻³ ~10 ⁻⁴
	27 L-氨基酸酶	NH ₃	10 ⁻² ~10 ⁻⁴
12 L-谷氨酸	28 谷氨酸脱氢酶	CO ₂	10 ⁻¹ ~10 ⁻⁴
13 L-精氨酸	29 精氨酸酶	NH ₃	5×10 ⁻⁴ ~5×10 ⁻³
14 L-赖氨酸	30 赖氨酸脱氢酶	CO ₂	10 ⁻¹ ~10 ⁻⁴

Table 1 Basic Properties of a Number of Enzyme Electrodes (2) Subject of Measurements (3) Enzyme (4) Detecting Electrode (5) Measurement Range (6) Glucose (7) Sucrose (8) Urea (9) Uric Acid (10) Cholesterolin (11) L-Amino Acid (12) Glutamic Acid (13) L-Arginic Acid (14) Lysine (15) Oxyglucose (16) Oxyglucose (17) Oxyglucose (18)&(19) Invertase + Multirotationase + Oxyglucose (20) Urease (21) Urease (22) Urease (23) Uricase (24) Cholisterinase + Oxycholisterinase (25) L-Amino Acid Enzyme (26) L-Amino Acid Enzyme (27) L-Amino Acid Enzyme (28) Glutamic Acid Dehydrogenase (29) Arginase (30) Lysine Dehydroynase

Following the development of enzyme electrodes, on the basis of single enzyme electrodes, one saw the development of double enzyme electrodes and even multi-enzyme coupled electrode systems. For example, α - amylase is used to diagnose acute pancreatitis and blockage of pancreatic ducts. One takes α - glucosamine enzyme and oxyglucose. Going through Glutard aldehyde, they are coupled and fixed on one side of a simple membrane of non-symmetrical acetic acid filaments. It was combined with electrodes that detect excesses of oxides of hydrogen, making a type of double enzyme electrode. Taking starch as the substrate, it is possible to measure, in serum, the α - starch activity and use it in outpatient chemical tests. Its basic reaction is



These electrodes are linear for the effects of α - amylase right up to 2500IU/dl. All of the measurements within 100 seconds are nominal. It is possible to measure over 1000 times. The correlation with standard methods for precision of analysis and degree of exactness is very good.

(II) Enzyme FET Sensors

Enzyme FET (field effect transistor) sensors are such that they add a layer of enzyme membrane on the insulation array or grid of ISFET that is sensitive to the material being measured. They make use of the biochemical reactions between this membrane and the material being measured and turn them into electrical signals. As a result of this, the amount of the material being measured is determined. Due to the fact that we have selected for use semiconductor techniques for the manufacture of these, it is possible to carry out extremely fine processing which enables one to produce miniaturized sensitivity components. It is also possible, on the same chip, to make components that are capable of measuring many types materials, realizing a change

to multi-functionality. Take the enzyme FET sensor monitoring for penicillin as an example. This sensor is structured from two ISFET capable of responding to pH. On an ISFET grid electrode, there is fixed a penicillin enzyme membrane. On a different FET grid electrode, there is no penicillin enzyme membrane included. One takes the sensor and soaks it in the test reagent solution. Penicillinase will take the penicillin and turn it into penicillin ketonic acid, causing a change in the pH value. When one monitors the difference in the electrode leakage currents of the two FETs, it is then possible to measure the concentration of the penicillin. This sensor, in the range of 0.2~25mmol penicillin, is capable of achieving linear responses. Enzyme FET sensors for urea and glucose as well as composite sensors for glucose and urea have all been reported. Most recently, Japan's NEC Kuriyama and others reported new methods involving membranes fixed as sediments on SOS (silicon extending outside of sapphires). Due to the fact that the background of SOS material is, itself, insulating, in solvents, when making measurements, it is possible not to consider the problem of current leakage. This makes the sealing and mounting technology much, much simpler. Moreover, if one evaporates a layer of metal in a thin layer on a surface where there is no externally protruding silicon to act as a simulated electrode for comparison and makes the comparison electrode and the FET combine together, in reality, they are unitized into a single component. At the same time, the SOS/ISFET mechanism has high strength. Its chemical stability is good. It is not sensitive to light. It is a relatively ideal material for the manufacture of biological FET sensors. It is made so that, as a urea sensor in 1~50mg/dl, and as a glucose sensor in 10~100mg/dl it shows linear responses. Response time is approximately 1 minute.

II. MICROBIOLOGICAL SENSORS

Microbiological sensor devices are formed by organizing and installing microbiological cells and electrochemical devices bonded with the carrier bodies. These have already been developed into two types of microbiological sensor devices. One type uses oxygen electrodes to test and measure the respiratory peculiarities of microbiological cells. One type takes as its foundation the detection and measuring of the metabolic products of microbiological cells.

As far as the composite systems of enzymes that microbiological forms make use of inside of cells are concerned, they take sugars, organic acids, amino acids or protein substances and turn them into various types of materials. Along with this, they take the energy that is obtained and use it for their own development and reproduction. As far as aerobic microorganisms are concerned, the result of their respiration of oxygen is that it causes oxides of organic acids to decompose, consuming oxygen gas and giving rise to carbon dioxide. It follows from this that it is possible to use O_2 electrodes or CO_2 electrodes to carry out electrochemical checks and measurements. In another respect, in the metabolic products of microorganisms, there are substances which are capable of being used for electrochemical checks and measurements, such as, glucose, sucrose, starch (amylose), propanoic acid, formic acid (methanoic acid), propyl (one character unclear) acid, and other similar substances, in order to measure the metabolic products for checks and tests.

It is very complicated to compare the biochemical reactions of microbiological sensor devices to the situation in enzyme sensors. Because of this, in order to measure subject materials selectively, it is necessary to pick subject materials that possess microbiological forms with special functions. When enzymes are not easy to obtain or difficult to purify, the use of the corresponding microbiological forms to make microbiological sensors is convenient. At present, use is already made of different types of microbiological material to launch the test production of glucose, methyl alcohol, ethyl alcohol, acetic acid, formic (methanoic acid), glutamic acid, lysine, the amount of oxygen biologically necessary (BOD), vitamin B, catalytic sensors, and other similar types of microbiological sensor devices. The principal properties of these are set out in Table 2.

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Particularly worth being brought up are microbiological BOD sensors. BOD is the most important quantity checked in measuring organic contamination. According to normal procedures, the measurements must be done at $20^{\circ}C$ with 5 days cultivation.

Moreover, they require quite high technical training. Recently, one has had the production of BOD sensor devices formed by the combining of alcohol enzymes taken and fixed onto a membrane of acetic acid fibers with oxygen electrodes. When the sample solution that contains the organic matter is poured into the sensor device, the organic compounds penetrate the fixed microbiological membrane. Along with this, it is unified with the microbiological material. The oxygen that is consumed is measured by the oxygen electrodes. The reaction times of these sensors vary with differences in the types of specimens. Acetic acid is 8 minutes. Glucose is 10 minutes. The smallest amount tested is 3mg/dm^3 . The life is 400 test iterations. The results match those from the 5 day method.

1 待测物	11 固定化微生物	12 检测电极	13 响应时间(分)	14 测量范围 mg/dm^3
2 葡萄糖	<i>Pseudomonas-Fluorescens</i>	O_2	10	2~20
3 甲醇	Unidentified Bacteria	O_2	10	5~200
4 乙醇	<i>Trichosporon Bacteria</i>	O_2	10	2~25
5 醋酸	<i>Trichosporon Bacteria</i>	O_2	10	3~60
6 谷氨酸	<i>Escherichia Coli</i>	CO_2	5	2~800
7 赖氨酸	<i>Escherichia Coli</i>	CO_2	5	10~100
8 吡啶素	<i>Saccharomyces Cerevisiae</i>	O_2	60	0.5~34*
9 烟酸	<i>Lactobacillus Arabinosus</i>	pH	60	10^{-5} ~5
10 吡啶素	<i>Citrobacter Freundii</i>	pH	10	100~500
BOD	<i>Trichosporon Cutaneum</i>	O_2	15	3~60

Table 2 Special Characteristics of a Number of Microbiological Forms
 (1) Substances to Be Tested (2) Glucose (3) Methyl Alcohol (4) Ethyl Alcohol (5) Acetic Acid (6) Glutamic Acid (7) Lysine (8) Anti-Mycetin (9) Nicotinic Acid (10) Tobocetin (11) Fixed Microbiological Form (12) Testing Electrode (13) Response Time (Minutes) (14) Measurement Range * The unit for measuring Anti-Mycetin was U/cm^3 .

III. IMMUNE SENSORS

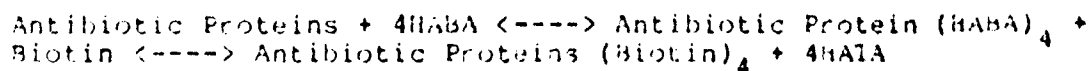
Immune sensors take the principles of immune measuring methods as their foundation and develop them into a type of biological sensor device. They are the product of a combination of the methods of immunology and the methods of electrochemical measurements. It is possible to divide them into the two types: non-marked immune sensors and marked immune sensors.

Non-marked immune sensor devices can take antibodies (or antigens) and fix them on the surface of a membrane. They can also take antibodies (or antigens) and fix them on the surface of metal electrodes. When forming antibody-antigen compounds, the density of the electric load on the membrane and the rate of ion migration in the membrane phase will both show changes. This leads to the electric potential of the membrane clearly varying. Use is made of the changes in these electrical signals to measure antigen and antibody reactions.

Marked immune sensor devices take radioactive isotopes, enzymes and other similar substances and uses them as marking agents. Take the enzyme immune sensor device for measuring insulin as an example. First, going through the compounding of oxyhydrogenase and insulin, one forms the enzyme marking antigen. Next, one takes fibrin and insulin antibodies and directly connects them to form an antibody fibrin or cellulose membrane. When one takes the antibody membrane and oxygen electrodes and combines them, one then forms an insulin immunity sensor. During measurements, the sensor is soaked in test fluid containing insulin and enzyme marked insulin. At this time, one takes enzyme marked insulin and non-enzyme marked insulin and, finally, combines them with the antibodies on the surface of the membrane, forming a compound body. One rinses the antibody membrane clean, eliminating the free ion antibodies and non-specific absorbed materials, as well as other similar substances. After that, one pours in a determined amount of H_2O_2 . Going through measurements of enzyme activity, it is possible to quantitatively measure the amount of insulin in the solution. The insulin sensors are capable of being used to measure the levels of insulin in blood fluids after doses of insulin are taken. As a result of this, one can derive the most appropriate dosages of the drug to use. Alpha fetal protein (AFP) is one type of tumor antigen. It has important significance in the

immune diagnosis of liver cancer. One type of specialized measurment, this antigen sensor is composed of a fixed antibody membrane and oxygen electrodes. The antibodies of anti-AFP are bonded covalently to a membrane made from (3) acetic acid fibers, 1.8 diamino-4 aminomethyloctane and Glutard aldehyde. Using hydrogen peroxide enzyme marking of AFP, one takes the sensors and the solution samples containing AFP and AFP marked with hydrogen peroxide and puts them into contact with each other. Along with this, they are maintained in this condition for 2 hours. After that, one uses 0.05 mol phosphoric acid buffer solution (pH 7.0) to rinse off the sensor. One takes it and places it into the phosphoric acid buffer solution and obtains the stable state electrical current associated with dissolved oxygen. One pours in 100 μ l of 30% H_2O_2 . Under hydrogen peroxide enzyme catalysis, oxygen gas is produced. On the basis of the corresponding electric current values, it is possible to solve for the amount of marking enzyme bonded on the membrane. As a result of this, one solves for the concentration of AFP. The sensitivity of this method is high. It is possible to measure 10^{-11} ~ 10^{-8} g/ml AFP. Responses are fast. Generally, within 30 seconds, one gets a stable state electric current. Using the same types of principles, people have already produced immune globulin G and human chorion promoting glandular hormone immune sensors.

On the basis of biological molecules possessing the unique special feature of identifying their own pattern of arrangement, there are also biological affinity sensors that have been presented to the public. For example, antibiotic proteins are a type of protein substance that is capable of bonding with biotin. They are also capable of bonding with the 2-(4-hydroxylbenzyl)nitrogen) benzoic acid compound (HABA). However, the affinity between antibiotic proteins and HABA bondings must be weaker than the affinity between them and other biotin bondings. Because of this, antibiotic protein-HABA compounds, given the existence of biotin, are very easy to dissociate, that is,



One takes HABA and hydrogen peroxide enzyme marked antibiotic proteins and forms a man made composite polymer membrane. This is matched with oxygen electrodes to make biotin sensors. When one takes sensor devices and soaks them in biotin solutions, they form a stable antibiotic protein-biotin compound. After going through rinsing, one pours in a fixed amount of H_2O_2 . The hydrogen peroxide enzyme that remains on the sensor breaks down and produces oxygen. From the oxygen, the electrodes make their checks. It follows from this that it is possible to solve for the concentration of biotin. Using the same type of principle, people have already produced insulin sensors.

IV. TISSUE SENSORS

Rechnitze reported the use of pig kidney tissue slices mounted on the surface of electrodes sensitive to ammonia gas to make biological sensors responding to glutamic acid amine. Kidney slices possess the activity of glutamine enzyme which would cause rapid metabolism of glutamine to generate ammonia and be detected by ammonia gas sensitive electrode. This sensor exhibits linear response in the range of 2×10^{-2} – 5×10^{-5} mol of glutamic acid amine and its slope, linear response range, detection limit can be kept unchanged for 28 days. The response time was 5 to 7 minutes and was better than the response of corresponding enzyme sensors. The upper skin tissue of frog has the ability of selectively permeate Na^+ and can be used to fabricate tissue sensor for monitoring Na^+ .

Biological sensors such as the enzyme sensors are mainly used in clinical test, fermentation industry, food industry, and environmental science studies. The determination of the constituent of body fluids such as blood or urine is an important

basis for clinical medical care. The content of glucose is closely related to diseases such as diabetes, tumor, and anaemia. The detection of urea can provide an important evidence for urinary system diseases, retardation of kidney function, and low blood pressure. Uric acid is a metabolic product of nucleic acid and nucleic protein and plays an important role in the detection of leukemia and increase of red blood cells. The cholesterol content in blood is closely related to hardening of artery and abnormality of heart muscle. Glutamic acid-propanic acid-amine transfer enzyme (GPT) and glutamic acid-acetic oxalate-amine transfer enzyme (GOT) are often used in detection of tuberculosis and heart problems. The biological sensors for these purposes have been realized and development is now emphasized on miniaturization, compacting, and multi-function applications. Glucose is the major source of carbon in the fermentation industry and the on-line detection of the glucose concentration is one of the most important factors in optimizing fermentation process. To produce monosodium glutamate from the fermentation method, the need for the sensor for glutamic acid is urgent. Similarly, the biological sensors for the detection of methane, acetic acid, vitamin, lysine can all be used in the fermentation industry. The application of biological sensors in the environmental protection and food inspection is also becoming wider. Except for BOD, organic phosphorous compound can be detected by butyryl cholinesterase electrode. The freshness of fish or meat can be detected by combining yellow oxygenizt. enzyme, phosphoric nuclease, and nuclease with oxygen electrode.

In inorganic analysis, using the antagonizing property of some of the ions against enzyme, minute quantity of F^- , Hg^{2+} , and Zn^{2+} and trace quantity of Cr^{3+} and Mn^{2+} can be detected. According to some report, enzyme electrodes can be used to detect more than 50 substances.

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